UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

JAN 16 1992

OFFICE OF
SOLID WASTE AND EMERGENCY RESPONSE

MEMORANDUM

SUBJECT: Region 6 Bioremediation Spill Response Plan

FROM: Karen Sahatjian, Chair
Implementation Subgroup, Subcommittee on National Bioremediation Spill Response

TO: Members of the Implementation Subgroup

Again, I would like to thank those members of the Implementation Subgroup who attended our meeting on Thursday, December 5, and Friday, December 6, 1991, in New Orleans, Louisiana. We have made great strides over the past few months in developing the Region 6 Plan and I sincerely thank everyone for their participation.

Attached for your review is the latest draft of the Plan. I would appreciate your careful attention in reviewing the draft, as I would like to present the Plan on behalf of the Subgroup in final form to the Regional Response Team next month. As we discussed at the New Orleans meeting, I have set up a conference call on January 28 to discuss any comments or concerns you may have regarding the Plan. Please call (202) 269-4246 at 2 p.m. Eastern time to be connected into the conference. The conference call has been scheduled for 2 to 3:30 p.m.

Please feel free to call me at (202) 260-1354 if you have comments or questions. Once again, thank you very much for contributing your time and effort to this important project.

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U.S. ENVIRONMENTAL PROTECTION AGENCY
REGION 6 BIOREMEDIATION SPILL RESPONSE PLAN

*** DRAFT - January 15, 1992 ***

SECTION 1

INTRODUCTION

Biodegradation is a natural process in which microorganisms (primarily bacteria and fungi) chemically alter and breakdown organic molecules into other substances -- such as fatty acids, carbon dioxide and water – in order to obtain energy and nutrient. The basis for this process is relatively simple: microorganisms require minerals and sources of carbon, as well as water and other elements, to survive and function.[1] The process can involve one step or a series of steps that proceed through the formation of molecules with successively fewer carbons.[2] Generally, the extent to which a particular organic molecule is biodegradable and the rate of degradation depend on the molecule's structural characteristics: chain length, amount of branching, number and arrangement of rings, and steriochemistry.[3,4,5]

Bioremediation is a treatment technology that utilizes biodegradation to reduce the concentration and/or toxicity of chemical substances such as petroleum products and other hydrocarbons. Because microbes capable of degrading hydrocarbons are commonly found in nature, most untreated hydrocarbon spills eventually are removed from the environment by microbial degradation and other processes. Bioremediation, however, seeks to accelerate natural biodegradation processes by applying specially chosen nutrients and/or microbes to spilled substances. Although microbes have been used extensively and successfully for many years to treat wastes and wastewater in controlled facilities, their potential as a tool for responding to spills of oil and hazardous substances in uncontrolled environments has only more recently received significant interest. (For additional information on bioremediation, refer to the list of references that follows Section 6 as well as Appendix A, which lists additional sources of information on bioremediation.)

This document presents a plan for considering and implementing bioremediation in response to spills in U.S. Environmental Protection Agency (EPA) Region 6. It was developed through the coordinated efforts of EPA's Subcommittee on National Bioremediation Spill Response, the Texas Water Commission, the Texas General Land Office, the Louisiana Department of Environmental Quality, the Office of the Louisiana Oil Spill Coordinator, and other members of the Region 6 Regional Response Team (RRT), using EPA's Interim Guidelines for Preparing Bioremediation Spill Response Plans.

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[1] Ralph J. Portier, "Bioremediation Using Adapted Bacterial Cultures, Topic 1: Examination of Site Data and Discussion of Microbial Physiology With Regard to Site Remediation," *Proceedings of the Hazardous Materials Control Research Institute's First National Conference on Bioremediation* (1990), p. 352.

[2] Ronald M. Atlas, "Microbial Degradation of Petroleum Hydrocarbons: an Environmental Perspective," *Microbiological Reviews* 45 (March 1981), p. 181.

[3] J.P.E. Anderson, "Principles of and Assay Systems for Biodegradation," *Biotechnology and Biodegradation*, vol. 4, Advances in Applied Biotechnology Series (Texas: The Portfolio Publishing Company, 1990), p. 131.

[4] Atlas, p. 181.

[5] Portier, p. 355.

1.1 PURPOSE

This document has a threefold purpose:

- To outline a process by which Federal On-Scene Coordinators (FOSCs) in Region 6 may request authorization to use bioremediation in response to spills of oil or hazardous substances (the authorization procedures presented are consistent with the National Oil and Hazardous Substances Pollution Contingency Plan (NCP));
- To define the types of information necessary to determine if bioremediation is feasible, provide as much of this information in advance as possible, and outline a mechanisms for capturing information on bioremediation use for future decision making; and
- To describe how to implement a bioremediation activity and determine if bioremediation is working.

The document is intended to guide decision makers in evaluating the appropriateness of bioremediation in the cleanup strategy for a spill and in undertaking a bioremediation activity. Ultimately, decisions regarding the use of bioremediation must be based on the FOSC's best judgment given the particular circumstances of the spill incident.

1.2 QUALITY ASSURANCE POLICY STATEMENT

[TO BE DEVELOPED BY THE REGION]

1.3 APPLICABLE FEDERAL AND STATE REGULATIONS

Legislation at both the federal and state level may affect decisions to use bioremediation. Existing regulations and policies that govern the use of bioremediation agents in responses to spills in Region 6 are summarized below.

1.3.1 Federal Regulations

At the Federal level, Subpart J of the NCP governs the use of chemical and biological agents -- which include bioremediation agents -- in responding to oil spills. Specifically, the Subpart:

- Restricts the use of chemical and biological agents that may affect U.S. waters to those listed on the NCP Product Schedule;
- Specifies technical product information that must be submitted to EPA for an agent to be added to the Schedule; and
- Establishes conditions for obtaining authorization to use chemical or biological agents in a response action.

For a bioremediation agent to be added to the NCP Product Schedule, the following technical product information must be submitted to EPA's Emergency Response Division in Washington, DC:

(1) Name under which the agent is sold;
(2) Name, address, and telephone number of the manufacturer or vendor;
(3) Name, address, and telephone number of primary distributors;
(4) Special handling and worker precautions for field application;
(5) Shelf life;
(6) Recommended application procedures, concentrations, and conditions for use;
(7) Efficacy data, including degradation rates and information on the test conditions; and
(8) Microbial content and percent composition by species (pathogenic organisms listed separately), optimal environmental conditions for use, and special nutrient requirements of the microbes, if applicable.

If EPA determines that the required data were submitted, EPA will add the agent to the Schedule. Note, however, that listing of an agent on the NCP Product Schedule does not constitute approval of that agent for use or confirmation of any claims regarding the agent's safety or effectiveness.

Data on agents listed on the NCP Product Schedule is available through EPA's Emergency Response Division in Washington, DC.

The FOSC, with concurrence of the EPA representative to the RRT as well as the RRT representative from the State with jurisdiction over the waters threatened by the spill, may authorize the use of any agent listed on the Product Schedule. When practical, the FOSC should consult with the Department of Commerce (DOC) and Department of Interior (DOI) representatives to the RRT before making a decision to bioremediate a spill. If the use of particular products under certain specified circumstances is approved in advance by the State, DOC, and DOI representatives to the RRT, and such pre-approval is specified in the Regional Contingency Plan, the FOSC may authorize bioremediation without consulting the RRT.

1.3.2 Regulations and Policies in the State of Texas

There are no regulations that specifically address the use of bioremediation for spill response in Texas. There are, however, legislative provisions prohibiting any activities that cause pollution of the State's waters (Texas Water Code, Chapter 26.121). Texas state agencies responsible for environmental regulation (including the Texas Department of Health, Railroad Commission of Texas, Texas Water Commission, Texas Parks and Wildlife Department, General Land Office and the Air Control Board) generally encourage the use of bioremediation for spill response when appropriate.

The Texas Water Commission (TWC), which has jurisdiction over hazardous substances and inland oil spills, encourages bioremediation and reviews proposals to use this technology on a case-by-case basis. Under the authority of Texas Water Code, Section 26.264 (e), the TWC is compiling a list of bioremediation agents for use in spill responses in Texas. Submission of the information required for agent listing by the TWC does not constitute approval to use an agent.

In some instances, a site-specific proposal may be required as a condition for bioremediation use on a spill site. Generally, for minor spills of petroleum products on land, TWC considers bioremediation to be an acceptable cleanup technology, provided that cleanup standards are met and water pollution does not result from application.

The General Land Office (GLO), which has jurisdiction over marine oil spills in the State of Texas, has not adopted specific policies regarding bioremediation for spill response. The Texas Oil Spill Prevention and Response Act of 1991 authorizes the Oil Spill Oversight Council to provide advice to the GLO on bioremediation-related issues.

1.3.3 Regulations and Policies in the State of Louisiana

The State of Louisiana has no regulations specifically restricting the use of bioremediation. However, Louisiana does require that selected oil spill cleanup methods be approved by the FOSC with concurrence from the Office of the Louisiana Oil Spill Coordinator. The Louisiana Oil Spill Prevention and Response Act of 1991 authorizes the Interagency Council to provide advice to the Office on bioremediation-related issues. Also, for all spills in the State, physical removal shall be the initial means of cleanup; bioremediation shall be considered only when physical means of cleanup have been exhausted or deemed unfeasible.

1.3.4 Regulations and Policies of Other States in Region 6

The remaining states in the Region are currently developing regulations and policies for bioremediation and spill response, which will be included in this section when available.

1.4 ORGANIZATION

The remainder of the plan is divided into five sections, which provide general guidance for bioremediation spill response:

- Section 2 describes issues relevant to managing responses involving bioremediation, including roles and responsibilities of various spill response agencies, and funding issues;
- Section 3 describes types of bioremediation agents and the process by which agents are evaluated and screened for possible use on spills;
- Section 4 contains assessment and implementation guidelines for the use of bioremediation in coastal and offshore waters;
- Section 5 contains assessment and implementation guidelines for the use of bioremediation in terrestrial environments [this section will be added at a later date]; and
- Section 6 outlines the components of a monitoring program for determining whether bioremediation treatment is breaking down the spilled substance into non-hazardous products without significant toxic effects.

These sections are followed by a series of appendices that include a Bioremediation Use Authorization Form, which should be used to record information to support decision-making, and a Bioremediation Use Follow-up Form, which will be used to maintain a historical data base on bioremediation activities. Taken together, these sections and appendices provide the framework and much of the necessary information to assess the appropriateness and feasibility of bioremediation in particular locations, evaluate and apply bioremediation agents, and monitor agent applications for safety and effectiveness.

1.5 REVISION PROCESS

The RRT will annually examine the information in this plan, considering any new advances in and additional experience with bioremediation, and revise the plan as appropriate.

SECTION 2

RESPONSE MANAGEMENT

[TO BE DEVELOPED BY THE REGION ACCORDING TO THE GUIDELINES BELOW]

This section discusses issues relevant to managing the response to a spill, with particular emphasis on managing a bioremediation activity. Because numerous response-related activities may be occurring simultaneously following a spill, which may be complicated by public and political pressure for swift and decisive action to mitigate spill impacts, the importance of having a well-defined design in place for initiating, coordinating, undertaking, and funding a bioremediation activity can not be understated.

2.1 RESPONSE STRUCTURE

The response structure or organizational framework identifies the participants (or titles of individuals and teams) in a response, their general areas of responsibility, and the lines of authority among them. A chart illustrating the participants in a bioremediation response activity in Region 6 and their inter-relationships would be very helpful in summarizing this information.

In developing this section, the following questions should be addressed:

- Who will manage the overall bioremediation activity?
- Who will be the likely participants (e.g., federal and state agencies) in the activity from the Region? What are their general roles?
- Who will be the likely participants, if any, from outside the Region? What are their general roles?
- Who will manage the monitoring portion of the activity?
- Who will develop an appropriate action plan for the bioremediation activity?
- Who will perform agent applications?
- Who will perform monitoring?
- Who will perform public outreach?

2.2 ROLES AND RESPONSIBILITIES

Describe in detail the specific roles and responsibilities of the likely participants in a bioremediation activity in Region 6. The information in this section should coincide with the information presented above on the regional response structure. The categories below may not be all inclusive.

2.1.1 Regional Response Team

2.1.2 Federal Agencies

2.1.3 State Agencies

2.1.4 International (U.S./Mexico)

2.1.5 Non-Governmental Organizations

2.1.6 Responsible Parties

2.3 NOTIFICATION AND ALERT PROCEDURES

Describe the procedures for notifying and alerting participants in a bioremediation activity in the Region. Include a description of how a bioremediation activity may be initiated.

2.4 RESPONSE RESOURCE ASSESSMENT AND ALLOCATION

Describe mechanisms or regional policy for determining whether regional resources available at the time of a spill incident are adequate for implementing a bioremediation activity. Describe how resources of regional participants, such as federal agencies, will be allocated in support of a bioremediation activity.

2.5 FUNDING

Money from the Oil Pollution Fund can be used to pay for those aspects of a bioremediation activity related to

- Removing spilled contaminants, and
- Monitoring for safety and efficacy in support of assessing or enhancing operations.

For example, monitoring in support of determining whether to reapply bioremediation agents is a legitimate expenditure. Monitoring specifically for research purposes, although important, is not a legitimate expenditure from the Fund.

Money from the Fund will cease to be available after the Federal On-Scene Coordinator determines that the response action is finished.

A policy statement from the National Pollution Fund Center regarding the eligibility of bioremediation related expenditures for payment from the Oil Pollution Fund is pending.

SECTION 3

BIOREMEDIATION AGENTS AND AGENT SELECTION

This section describes the various types of bioremediation agents, a procedure for evaluating them, and guidelines for selecting the appropriate agent for use in a particular spill situation.

3.1 BACKGROUND

Section 311 of the Clean Water Act requires that the U.S. Environmental Protection Agency (EPA) prepare a schedule of dispersants and other chemicals that may be used in preparing for and responding to discharges of oil and releases of hazardous substances, as provided for in the National Oil and Hazardous Substances Pollution Contingency Plan (NCP), 40 CFR Part 300. This schedule is known as the NCP Product Schedule. The Schedule lists agents that may be authorized for use on oil discharges in accordance with the procedures set forth in Section 300.910 of the NCP. (Authorization of use requires that the Federal On-Scene Coordinator (FOSC) considering the use of a dispersant or other agent, such as a biodegradation enhancing agent, seek the concurrence of the Regional Response Team prior to the agent's application.) Any agent considered for application to an oil spill should be listed on the NCP Product Schedule.

The NCP Product Schedule currently divides agents into five categories: (1) dispersants, (2) surface collecting agents, (3) biological additives, (4) burning agents, and (5) miscellaneous oil spill control agents. Most bioremediation agents, including those that are solely nutrients, are listed as biological additives, as the designed purpose of these agents is to enhance the rate of oil biodegradation by increasing microbial activity. There are also bioremediation agents listed as dispersants; these agents are water-based products that claim to enhance the rate of oil biodegradation by emulsifying spilled oil thereby making it more "bio-available." Additionally, other products that do not fit a current regulatory definition because of their unique nature may be listed as miscellaneous agents. Use of any of these agents should be consistent with the Regional Response Team's general guidelines for their application and use.

3.2 TYPES OF AGENTS

The number and type of agents which claim to enhance the rate of biodegradation has broadened to fill the current perceived market. Although there are no current regulatory definitions for every type of bioremediation agent, the following are broad definitions for currently available agents:

Microbial Agents -- concentrated cultures of oil-degrading microorganisms grown on a hydrocarbon-containing media that have been air- or freeze-dried onto a carrier (e.g., bran, cornstarch, oatmeal). In some cases, the microorganisms may be grown-up in bioreactors at the spill site. All commercially available agents use naturally-occurring microorganisms. Some agents may also contain nutrients to assure the activity of their microbial cultures. This type of agent is intended to provide a massive inoculum of oil-degrading microbes to the affected area thereby increasing the oil-degrading population to a level where the spilled oil will be used as a primary source of food or energy. Microbial

agents are designed to enhance the biodegradation of oil at any location and would be most useful in areas where the population of indigenous oil-degraders is small.

Nutrients -- agents containing nitrogen and/or phosphorous as the primary means to enhance the rate of growth of indigenous oil-degrading microorganisms. This type of agent is intended to increase the oil-degrading biomass already present in an affected area to a level where the oil will be used as a primary source of food or energy. Because the natural environment may not have sufficient nutrients to encourage bacterial metabolism and growth, extra nutrients may be required. The purpose of this type of agent, therefore, is to provide the nutrients necessary to maintain or increase microbial activity and the natural biodegradation rate of spilled oil. This type of product has been used in Prince William Sound, Alaska and Prall's Island, New Jersey to reduce the amount of oil on contaminated beaches.[6]

Enzymatic Agents -- bio-catalysts designed to enhance the emulsification and/or dispersion of oil and make it more available to microorganisms as a source of food or energy. These agents are generally liquid concentrates, which may be mixed with surfactants and nutrients, that are manufactured through fermentation. This type of agent is intended to enhance biodegradation by indigenous microorganisms. ~~Can be used~~ OSE II & can be used as a first response & only response tool

Other Agents -- include agents that do not fall under the above definitions, such as application mechanism agents that are designed to have an affinity for oil and bring together the elements needed for enhanced oil degradation. Examples of application mechanism agents include time release capsules, liposomes, timed-release fertilizers (e.g., custom blend), and agents that make oil more hydrophobic.

3.3 AGENT EVALUATION PROCEDURE[7]

In considering bioremediation agents listed on the NCP Product Schedule or proposed by agent vendors for potential use in spill cleanup, it is important that response decision-makers evaluate the various characteristics of agents, particularly their safety and efficacy. From the perspective of planning for bioremediation use, the most appropriate time to evaluate agents -- whether performed by EPA, product vendors, or contractors -- is before a spill occurs. Provided below is a procedure designed specifically to aid in such an evaluation, which is directed ultimately at identifying bioremediation agents that will be safe and effective in field applications. There

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[6] For information on uses in Alaska, refer to Pritchard and Costa's article entitled "EPA's Alaska Oil Spill Bioremediation Project" in *Environmental Science & Technology* (Vol. 25(3), 1991), and the article by Chianelli *et al.*, entitled "Bioremediation Technology Development and Application to the Alaskan Spill" in *Proceedings: 1991 Oil Spill Conference.*

[7] The procedure described in this subsection for evaluating bioremediation agents is based on draft protocols developed by a panel of recognized national experts from industry, academia, and government at the request of EPA. The panel was brought together under the auspices of the University of Pittsburgh Trust's National Environmental Technology Applications Corporation (NETAC), which has been working with EPA on the development of bioremediation agent screening protocols since November 1989. A full description of the development process may be found in the NETAC panel's "Oil Spill Bioremediation Product Testing Protocol Methods Manual." As of this writing, the protocols were still under development.

may be circumstances, however, under which there is not adequate time to perform thorough agent evaluations before a decision regarding bioremediation use must be made. In these instances, the procedure below should be used as a guide to determine whether existing information on individual agents is adequate to support further consideration of their use.

The procedure follows a "tiered" approach (a "Base Tier" and four subsequent tiers) whereby bioremediation agent performance data is gathered as a means to predict the safety and efficacy of agent applications in various field settings or habitats where oil spills may occur. The proposed procedure is intended as a standard methodology for assessing the effectiveness and safety of different bioremediation agents. Following the procedure will not assure that a tested agent will be effective in spill cleanup; however, following the procedure should increase the level of confidence that use of an oil spill bioremediation agent will be effective and safe.

3.3.1 Base Tier -- "Go"/"No Go" Requirements and Information

Information on a bioremediation agent should be collected from the agent vendor and an initial screening of the information performed. Objectives of this screening are to:

- Obtain basic information on a bioremediation agent's makeup;
- Ensure satisfaction of minimal regulatory approvals that may be required;
- Certify whether the agent contains pathogenic, carcinogenic, or hazardous substances or microorganisms normally considered unacceptable for release into the environment; and
- Ensure that the agent is listed on EPA's NCP Product Schedule.

Information needed from the agent vendor to perform this initial screening includes the agent's exact chemical and biological makeup as well as formulation characteristics, and proof of the agent's listing on the NCP Product Schedule.

3.3.2 Tier I -- Feasibility Assessment

Additional vendor information on a bioremediation agent should be collected to support an assessment of whether use of the agent is feasible. The objectives of this tier and assessment are to obtain an understanding of a vendor's capabilities; an agent's availability, contents, and proposed method of use; and an agent's history of use, where applicable. Agent information needed from the vendor to perform this assessment includes the following:

- Application rates and methods;
- Mode of biodegradation and calculated efficiency;
- History of use at previous cleanups;

- Chemical properties, fate and persistence, and potential toxicity or bioaccumulation for humans, mammals, and birds based on a review of published literature and chemical databases;

- Acute or chronic toxicity to one marine or freshwater fish and invertebrate species selected from U.S. EPA's "Effluent Monitoring Program;" and, where available,

- Effectiveness in enhancing biodegradation over a baseline standard or control demonstrated by descriptions and quantitative analytical results of any laboratory or field studies performed (such as results of gas chromatographic analyses of treated and untreated samples for alkanes and/or aromatics).

A description of the management structure and qualifications of the vendor's organization is also needed.

3.3.3 Tier II -- Laboratory-Scale Data

Standard laboratory methods should be used to develop data on the an agent's toxicity and its ability to stimulate the biodegradation of a standard oil. The specific objectives of this tier are to evaluate the relative ability of a bioremediation agent to degrade oil, or stimulate the rate of biodegradation, under defined and controlled laboratory conditions and to determine the potential toxicity associated with the agent's use through the performance of standard toxicity tests. Analytical methods developed by EPA should be used to perform these laboratory studies.

The approach to evaluate an agent's relative effectiveness at degrading oil should:

- Provide sufficient information to indicate with a firm degree of confidence that the agent is degrading oil constituents;

- Provide an indicator of total microbial activity; and

- Assure the viability of the culture being tested, where applicable.

The approach should include temperature, salinity, and nutrient testing to document the conditions under which an agent's ability to degrade a standard type of oil was determined.

The approach to evaluate an agent's toxicity should be conducted for specific fresh-water or marine species on the agent alone and the agent and standardized oil combined. Seven-day chronic estimator methods should be performed using daphnia (Ceriodaphnia) and fathead minnows (Pimephales) for fresh water, and mysids (Mysidopsis) and silversides (Menidia) for marine applications. These are standard tests; additional tests specific for Regional species may be desirable. The mammalian toxicity of agent constituents should be reviewed in existing data to determine whether any special precautions need be taken with regard to application methods, rates, or timing to protect persons applying agents as well as indigenous wild life.

3.3.4 Tier III -- Simulated Field Test Demonstration

Based on findings of previous tiers, microcosm systems should be used to perform simulated field test demonstrations on a bioremediation agent, as appropriate. The objective of this tier is to predict a bioremediation agent's effectiveness at degrading oil or petroleum products in specific field settings or habitats.

Although EPA-approved microcosm systems for performing simulated field test demonstrations are still under development at the time of this writing, the approach for performing these tests is to use microcosm systems that simulate actual biodegradation field kinetics. This approach will aid in determining the relative effectiveness and toxicity of an agent under conditions that cannot be modeled in standard laboratory methods, such as those proposed in Tier II of the procedure. Microcosm systems that should be considered for simulated field test demonstrations of agents include: (1) cobble beaches, both marine and fresh water; (2) open water, both marine and fresh, warm and arctic; (3) marshes and wetlands, both marine and fresh water; (4) inland shoreline; (5) sandy beaches, both marine and fresh water; and (6) land/soil.

3.3.5 Tier IV -- Limited Field-Scale Demonstration of the Agent

Depending on the results of the simulated field test demonstration in Tier III, a limited field-scale demonstration of a bioremediation agent should be conducted. The objectives of this field demonstration are to test the effectiveness and toxicity of the bioremediation agent in actual field tests and to verify the accuracy of Tier III laboratory results in predicting field efficacy using the actual field monitoring data obtained. The approach for performing these demonstrations is to collect information during active field testing to support an evaluation to confirm the bioremediation agent's estimated environmental safety and efficacy.

At this time, EPA-approved protocols for performing limited field-scale demonstrations in various settings are still under development. Until such protocols become available, the guidelines provided in Section 6 for monitoring field applications of bioremediation agents could be used for evaluating limited field-scale demonstrations of agents.

3.4 AGENT SELECTION

Primarily because of a lack of specific bioremediation agent research and agent testing standards; the selection of a bioremediation agent that will enhance the rate of oil biodegradation is currently more of an art than a science. For most of the bioremediation agents currently on the NCP Product Schedule, there is only limited comparative data by which to measure their relative efficacy and safety. Some of the agents have been tested by EPA according to the procedure described above; however, these agents are not necessarily better than ones that have not been tested by these methods. Therefore, agent selection will remain largely a subjective process until a larger and more complete database of standard test data on agents can be assembled.

To the extent possible, the selection of bioremediation agents for potential use in oil spill cleanup against specific oils or petroleum products should take place in anticipation of an oil discharge, when time is not a critical factor. For areas where the potential for an accidental spill are high or where there has been a high frequency of spills (assuming the use of bioremediation

agents is allowed in these areas), specific plans should be developed that outline the most likely petroleum products to be spilled and the alternative bioremediation agents that could be used to perform cleanup of those products in these areas.

In selecting a specific bioremediation agent for use in a particular spill situation or scenario, there are a number of questions that should be answered; these are provided below. These questions reflect the process to select a specific bioremediation agent, which assumes that bioremediation use is not prohibited in the spill location and that bioremediation is a feasible method for treating the spilled contaminant. The questions focus decision makers on evaluating the availability of a bioremediation agent and the resources necessary to support its use, as well as an agent's efficacy and safety. Answers to these questions should be documented on the Bioremediation Use Authorization Form contained in Appendix B and described briefly in Section 4.1.2.

For a particular spill incident:

(1) What are the characteristics of the spill environment?

- Is it an open water, cobble beach, sandy beach, marsh, wetland, or other location?
- Is it a marine or fresh water environment?
- Have spills occurred in the location in the past?

(2) What are the characteristics of the spilled oil?

- What specific oil or petroleum products are involved?
- What are the physical attributes of the oil (e.g., heavy, sticky, volatile)?
- Is the oil weathered (i.e., older than 48 hours)?

(3) Has the spilled oil or petroleum product(s) ever been treated by bioremediation?

- If so, what type of agent or specific agent was used in each instance?
- What were the conditions for use?
- Was a significant level of degradation measured (based on oil chemistry and microbial tests) that could be attributed to the treatment?

For each agent proposed or considered for application:

(4) Is the agent listed on the NCP Product Schedule? Does it contain a known pathogen?

(5) To what Tier-level equivalent has the agent been formally evaluated?

(6) Does the agent have any corroborated [laboratory or field] data indicating that it enhances biodegradation and that it should be safe to use in the affected spill environment?

(7) Has the agent been used on previous oil spills?

- What were the characteristics of the oil and the spill environment in each instance?
- Was a significant level of degradation measured (based on oil chemistry and microbial tests) that could be attributed to use of the agent?
- Is a reference available? (If so, make contact.)

(8) What is the recommended application rate for the agent (per gallon of oil) and how frequently would it need to be re-applied? ← for other products per gallon and area covered

(9) Is sufficient quantity of agent available to adequately treat the area of the spill where the agent will be applied, including any re-applications if necessary?

(10) Does the agent vendor have large-scale application capability or access to such capability?

(11) Is there sufficient contractor support to assist with the initial application and any re-applications if necessary?

(12) What precautions would need to be taken to reduce the likelihood of improper agent handling and application as well as adverse effects related to the application procedure?

← the most exact measurement of how much oil is contained in a given area and how much more area could this oil potentially cover and how thick is it on the surface of the water.

SECTION 4

ASSESSMENT AND IMPLEMENTATION FOR COASTAL AREAS, ESTUARIES, AND OFFSHORE WATERS

This section provides guidelines for assessing the feasibility of bioremediation for spill response and for developing action plans to implement bioremediation treatment in coastal areas, estuaries, and offshore waters. The section is organized in three parts: (1) a general discussion of decision tools, including a decision process and a use authorization form to assist Federal On-Scene Coordinators (FOSCs); (2) bioremediation feasibility assessment criteria; and (3) implementation directions.

4.1 DECISION TOOLS

Spills may be good candidates for bioremediation treatment based on characteristics of the spill and environmental sensitivities of the spill location. To assist FOSCs and the Regional Response Team (RRT) in evaluating spills for bioremediation treatment and to document the basis for response decision making, the following two tools are provided: (1) a diagram outlining the decision process that FOSCs should follow when deciding whether to use bioremediation; and (2) a form for obtaining authorization to use bioremediation that specifies the information that should be collected at the time of a spill.

4.1.1 Decision Process

Decisions to use bioremediation should be made after applicable regulatory policies, potential environmental impacts, operational feasibility, logistical coordination, and other pertinent issues have been evaluated. The process to determine whether bioremediation may be feasible for a particular spill is illustrated in the decision process diagram in Exhibit 4-1. Details for addressing the specific issues outlined in the decision process are provided below in Section 4.2, "Feasibility Assessment Criteria."

4.1.2 Bioremediation Use Authorization Form

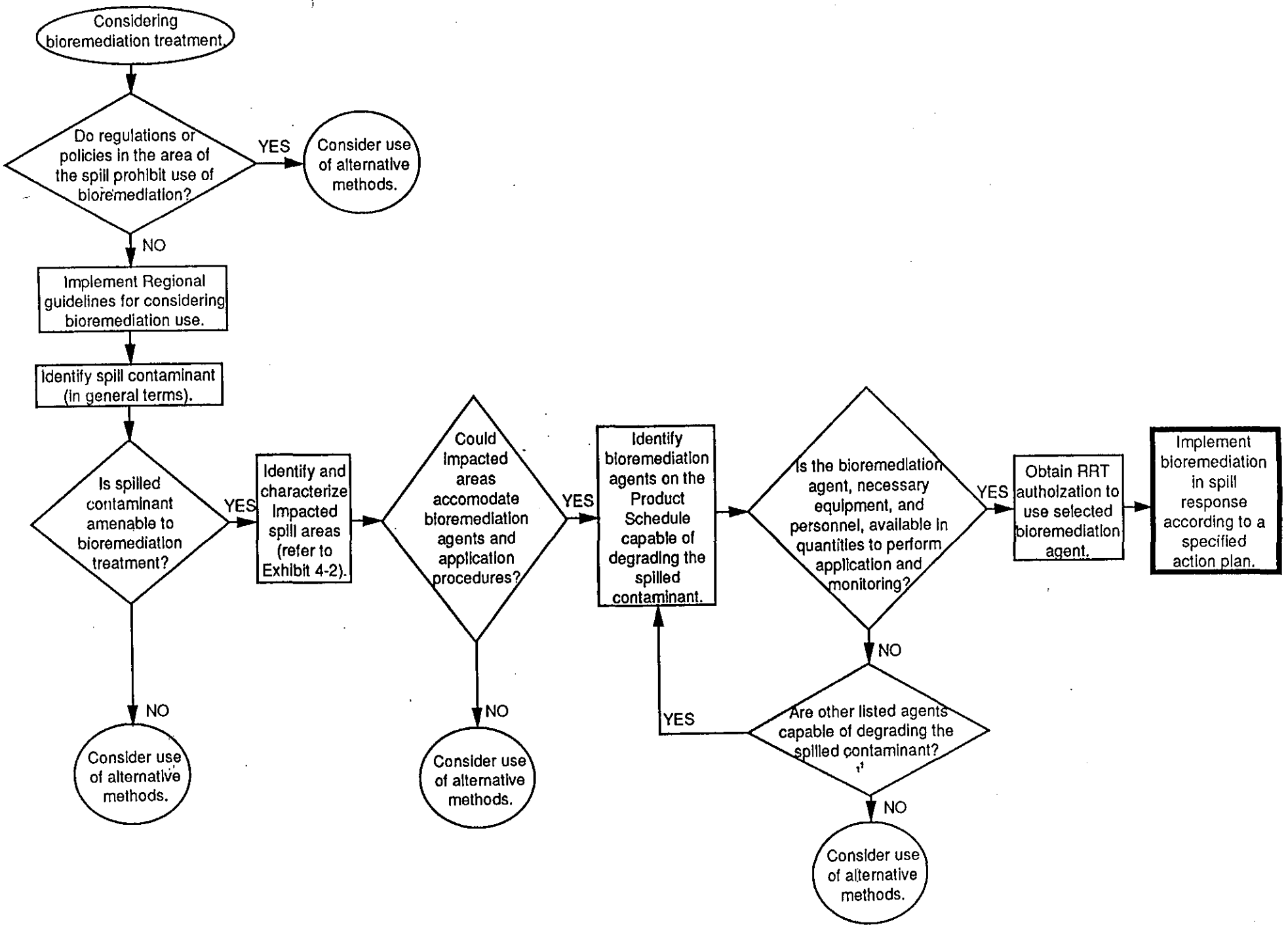
A Bioremediation Use Authorization Form that specifies the minimum information requirements necessary to support decisions regarding the use of bioremediation is included in Appendix B. The form requests information such as the time, date, and location of the spill; contaminant type; and current as well as projected weather conditions. Once the form has been completed, it should provide pertinent information needed to make decisions regarding the use of bioremediation.

A completed authorization form will be transmitted to the RRT for the required authorization to proceed with bioremediation treatment. The RRT shall approve or disapprove the use of bioremediation within 24 hours of receiving a completed Bioremediation Use Authorization Form from an FOSC.

4.2 FEASIBILITY ASSESSMENT CRITERIA

Assessing the feasibility of bioremediation is basically a two-stage process. The first stage determines whether a particular spill is a candidate for bioremediation treatment. The second

EXHIBIT 4-1
BIOREMEDIATION FEASIBILITY ASSESSMENT PROCESS



stage determines whether bioremediation can be implemented effectively, given the characteristics of the spill situation and the availability of needed equipment and personnel.

4.2.1 Incident Characteristics

The characteristics of a spill incident provide indications of the extent to which bioremediation treatment will be safe and effective against the contaminant spilled in a particular location. To aid in assessing bioremediation as a possible response option in several different habitats, a bioremediation advisability matrix has been provided in Exhibit 4-2. The matrix provides general guidelines regarding the advised use of bioremediation in different marine habitats based primarily on concerns for preserving habitats and minimizing harm to the indigenous flora and fauna. other than water intake, there is no restriction on area OSEI will not help in the advisability matrix in exhibit 4-2

Characteristics of Spilled Oil OSEI has also been used in all these other areas as of 4-30-2014

Before consulting the matrix, the possibility and practicality of using bioremediation against the type of oil or petroleum product spilled should be evaluated. That is, the extent to which the chemical constituents of the spilled oil (which characterize that oil) are biodegradable and/or toxic to microorganisms needs to be assessed before bioremediation treatment is considered further.

Generally, the constituents of oil can be divided into four categories of hydrocarbons: (1) saturates (which includes *n*-alkanes, branched alkanes, and cycloalkanes); (2) aromatics; (3) asphaltenes; and (4) resins.[8] Rates of biodegradation are typically the highest for *n*-alkanes, followed by branched or cycloalkanes and then low-molecular-weight aromatic compounds; rates for high-molecular-weight aromatic compounds, asphaltenes, and resins tend to be very low.[9,10] → their toxicity tends to be low as well In addition, many of the low-molecular-weight saturated and aromatic compounds are highly volatile, tending to evaporate quickly, as well as toxic to microorganisms and other forms of life. → OSEI has tested effectively at remediating these as well

Some general characteristics of various types of petroleum products are presented below.

Gasolines are typically mixtures of hydrocarbons containing 4 to 12 carbon atoms. Gasolines may evaporate completely in a few hours under temperate conditions. They pose a risk of fire and explosion associated with their high volatility, and are generally more toxic than crude oil because of their high concentration of low-molecular weight compounds. Because of their potential toxicity to microorganisms and high volatility, gasolines and other very light oils may not be good candidates for bioremediation. → OSEI has proven to eleminte the fire hazard associated with fuels

Kerosenes, often used for jet fuel, primarily contain hydrocarbons with 10 to 16 carbon atoms and tend to evaporate relatively quickly. Kerosenes and other light oils can

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[8] R.M. Atlas, "Biodegradation of Hydrocarbons in the Environment," *Environmental Biotechnology* (New York: Plenum Press, 1988), p. 214.

[9] Atlas.

[10] J.G. Leahy and R.R. Colwell, "Microbial Degradation of Hydrocarbons in the Environment," *Microbiological Reviews*, September 1990, p. 305.

EXHIBIT 4-2

BIOREMEDIATION ADVISABILITY MATRIX

Habitat Type	Bioremediation Treatment		
	Preferred	Optional	Not Advisable
Bay Margins		X	
Dredge Spoil Banks		X	
Endangered Species Habitats		X	
Erosional Scarps		X	
Exposed Tidal Flats	X		
Mangroves	X		
Marshes	X		
Open Sand Beach		X	
Open Water/Offshore		X	
Oyster Reefs/Shellfish Beds			X
Riprap/Man-made Shore		X	
River Banks		X	
Sand/Shell Beach		X	
Seagrass Beds	X		
Sheltered Tidal Flats	X		
Tidal Inlets		X	
Water Intakes			X

generally be considered as potential candidates for bioremediation.

Diesel fuels and **bunkering fuels** contain a greater percentage of hydrocarbons with increasing numbers of carbon atoms. They also contain greater proportions of substances other than hydrocarbons. They have low volatilities and persist in the environment for an increasing period of time as the "weight" of the material increases. These medium to heavy oils can generally be considered as potential condidates for bioremediation, however, they have been reported as less susceptible to biodegradation than lighter oils, particularly if they are weathered.[11] OSER has numerous tests proving its ability to reduce PAH's

Characteristics of Affected Habitats

After evaluating the spilled oil's susceptibility to biodegradation, the habitats most likely to be impacted by the spilled contaminant should be identified and characterized. If possible, the affected areas should be classified according to the habitats listed in the bioremediation advisability matrix in Exhibit 4-2. Descriptions and typical characteristics of these habitats can be found in the Texas Water Commission's, *Coastal Region Spill Response Map Support Data* (August 1989 version). Decision makers should then refer to the advisability matrix to determine whether bioremediation is a "preferred," "optional," or "not advisable" treatment alternative within the impacted habitats. The additional guidelines below regarding the use of bioremediation in marshes, beaches, and offshore/open waters also should be reviewed.

Marshes: Because trampling associated with mechanical cleanup methods is physically disruptive and may exacerbate the adverse effects of oil in these areas, mechanical cleanup is not recommended for spills affecting marshes and wetlands. In these habitats, bioremediation or "no action" may be among the most appropriate response alternatives.

Beaches: For lightly or moderately oiled beaches, particularly where mechanical cleanup may cause more environmental harm than untreated oil, or where mechanical cleanup may be logistically impossible, bioremediation or "no action" may be the most effective principal response alternatives. For heavily oiled beaches, bioremediation may be inappropriate as a principal response method, but could be considered in concert with other cleanup methodologies.

Offshore/open waters: Generally, because there is limited evidence regarding its effectiveness in treating spills in open waters and because techniques for monitoring spills in offshore waters are currently not well developed, bioremediation should not be used as the principal response method for open water spills. This is not true for OSER on fresh, salt or brackish water spills. thousands of spills have been cleaned upon open water and on water in BAYS as of 4-30-2011

4.2.2 Logistical Concerns

Characteristics of a spill incident, including characteristics of affected habitats, should determine whether a spill is a candidate for bioremediation treatment. If, based on these factors, bioremediation has not been eliminated as a response alternative, then the logistical feasibility of implementing an appropriate bioremediation action plan should be evaluated. Implementation

[11] Ronald M. Atlas, "Microbial Degradation of Petroleum Hydrocarbons: an Environmental Perspective," *Microbiological Reviews*, March 1981, p. 182.

considerations include the proposed scale of a bioremediation activity, the availability of the bioremediation agent (or agents) proposed for application, and the availability of the resources necessary to conduct the application method and monitoring recommended for the agent or agents proposed for use in each affected habitat. (The latter two considerations are highly dependent on the first.) The OSEI Corporation has enough OSEII in a warehouse to address 1,000,000 gallon spills, and can produce

Scale of Bioremediation Response OSEII faster than it can be applied. 4-30-2011

The first step in assessing the logistical feasibility of bioremediation is to determine the scale of the bioremediation response. The scale of the bioremediation response refers to the extent to which bioremediation will be involved in the cleanup, particularly in terms of the size of the application area and the possible need for conducting a limited field-scale demonstration of an agent. The scale of the bioremediation response effort could determine the amount of agents, the number of personnel, and the equipment resources necessary to complete agent application and monitoring of the bioremediation response effort. OSEII should be used at all time frames in all impacted area's - there should be no restriction to

Agent Availability area or point of time, all windows are acceptable.

Once the proposed scale of the bioremediation response activity has been determined and agent alternatives have been identified (refer to Section 3 for agent evaluation and selection guidelines), the availability of these agents for use at the spill location should be assessed. If an agent is not available in quantities necessary to complete the bioremediation response activities, the scale of the bioremediation response should be reevaluated, a different agent should be considered, or bioremediation should be eliminated as a response alternative. OSEII is and has been used as a first and only response tool.

Application and Monitoring Resources

Several application methods are generally available for bioremediation agents and each method may have unique resource requirements for its implementation. To determine whether requirements for application methods will preclude or limit the use of a particular method, the habitat(s) where bioremediation is being considered for cleanup should be evaluated to determine which method is most appropriate. For example, because offshore spills often require treatment over a large area, fixed-wing aircraft or helicopters equipped to apply bioremediation agents may be used as they provide the most efficient application methods for treatment of large areas. any type of spray apparatus can be

Next, the types and supply of available equipment and personnel adequate to implement and monitor the bioremediation response effort, as well as access to laboratory facilities for sample analyses, should be evaluated. (Refer to Section 6 for recommended monitoring activities and proposed monitoring resource requirements. See Appendix C for a listing of personnel and equipment resources in Region 6; see Appendix D for a listing of laboratory facilities in Louisiana and Texas that can perform monitoring tests.) If the desired bioremediation response requires more resources than are currently available or attainable, the scale of the bioremediation response may need to be reduced.

4.3 IMPLEMENTATION

Before initiating bioremediation treatment in spill response, several steps should be completed. First, the FOSC shall notify the RRT that the use of bioremediation is being

proposed by transmitting the completed Bioremediation Use Authorization Form. Second, a specific plan of action should be developed, which addresses issues necessary to ensure an efficient and effective bioremediation spill response.

4.3.1 Notification Process

After finalizing the selection of a bioremediation agent, or agents, and the appropriate application method for each affected habitat proposed for treatment, the completed Bioremediation Use Authorization Form should be transmitted to the RRT for concurrence with the decision. If use of bioremediation in the spill area has been pre-approved or pre-authorized by the RRT, this concurrence is not necessary; however, the FOSC must still notify the RRT of the decision to use bioremediation.

4.3.2 Action Plan

Action plans are important to ensure the safe, coordinated, and well-documented implementation of bioremediation treatment. Action plans are comprised of systematic procedures and guidelines that clarify and resolve issues such as worker and public safety, documentation requirements, response personnel roles and responsibilities, agent application protocols, and application control and oversight considerations. Complete action plans must include spill- and site-specific considerations. The primary elements that should be included in all bioremediation action plans are described below. An outline of an action plan is provided in Appendix E.

Public Safety

Public safety is paramount in any bioremediation project. The following actions should be taken during spill response to ensure public awareness and protection:

- Provide news releases and updates to newspapers, radio, and television stations, as well as neighboring areas that could be potentially impacted by bioremediation activities;
- Address safety issues regarding potential hazards of ground transport of bioremediation agents through populated areas; and There are no dangers with OSEI, small amounts have been ingested without adverse effects and you can safely wash your hands with OSEI
- Thoroughly analyze all weather data to ensure that aerial applications of bioremediation agents will not drift into commercial, industrial, and residential areas.

Site Safety

When handling bioremediation agents, the following guidelines should be practiced to avoid unnecessary injury:

- Unload, mix, and reload a bioremediation agent into application equipment in accordance with the manufacturer's suggested procedures and applicable OSHA requirements;

- Provide sorbents and containers at all bioremediation staging areas to allow for rapid cleanup of agents that may be inadvertently spilled; [illegible] spills can be cleaned up & used & residual effluent washed
- Periodically inspect all bioremediation staging areas to ensure compliance with all OSHA safety regulations; and

- Investigate accidental spills of bioremediation agents that occur during the bioremediation activity to determine if operational procedures could be modified to avoid future accidents of the same nature.

To avoid disturbances to the treated area after bioremediation application, all treated and control sites should be secured. The following steps are necessary to establish an effective security plan: ~~OSHA's initial action are quick and an area would~~

- To avoid potential injury, post warning signs or secure the treated area to differentiate the site from surrounding localities; and no potential enjury even the oil itself present
- Guard the site 24 hours a day, preferably by staggering worker shifts among the different response agencies to protect the site without having to incur the additional expense of hiring guards. really not necessary

Worker Safety

All bioremediation agents should be treated with caution. Because spilled oil is considered a hazardous waste under the Operational Safety and Health Administration (OSHA), all workers involved in oil spill response will be subject to all OSHA and U.S. Environmental Protection Agency (EPA) regulations.

At least 1 person on the team should have completed the OSHA 48-hour training course; all other team members should have completed the OSHA 24-hour training course.

All workers involved with the physical movement, transport, mixing, application, or monitoring of bioremediation agents, as well as individuals involved in the repair/washing of application equipment, shall wear proper protective clothing. Personal protection gear should be identified in a site application plan; required clothing and equipment should be determined by the site-safety officer. no special clothing required at any point, if wind is high safety glasses should be worn

Safety guidance shall be provided to all workers concerning dangerous wildlife in the area (e.g., alligators, snakes), harmful environmental conditions (e.g., over-exposure to sun, water dangers), and operating procedures for bioremediation application equipment (e.g., boats, fixed-wing aircraft).

Documentation

Throughout the response period, all implementation procedures and subsequent outcomes should be documented. Documentation should include video footage, photographs, and a daily written log. At a minimum, the documentation log should contain the following details:

- Amounts and types of bioremediation agents applied;
- Frequency and method of application;
- Number of personnel involved in response;
- Weather;
- Equipment used; and
- Observed effects of application of bioremediation agents on spilled oil.

Agent Application Protocols

Bioremediation agents can be applied by land, air, or sea. The particular details of the spill, particularly the impacted habitats and current and forecasted weather conditions, will determine the most effective and appropriate application method. Suggested application protocols should be provided by the contractor or response team.

Recommended requirements for monitoring the effectiveness and safety of a bioremediation agent application are described in Section 6.

SECTION 5

ASSESSMENT AND IMPLEMENTATION FOR TERRESTRIAL ENVIRONMENTS

[TO BE DEVELOPED BY THE REGION]

SECTION 6

MONITORING

Bioremediation is often assumed to enhance the biodegradation of oil or hazardous substances without increasing adverse impacts to human or ecological health. Until there is evidence to confirm this assumption, however, bioremediation effectiveness and safety need to be monitored through a sound program of applied science.

This section presents a general plan that provides Federal On-Scene Coordinators (FOSCs) with the information needed to prepare for, implement, and oversee monitoring activities designed to enable the objective evaluation of bioremediation in the response to a spill. (Please note that these activities should be coordinated with the activities of the Shoreline Cleanup Assessment Team.) The plan is intended primarily for monitoring oil spill bioremediation activities conducted in coastal areas and estuaries. Preparing for monitoring in advance of a spill is stressed in order to reduce the number of decisions that must be made during an actual response, increase the effectiveness of monitoring efforts, and promote the use of standard, accepted protocols.

The plan presented in this section is intended to be implemented primarily through the responsible party's or the FOSC's contractor. The contractor will use this plan as a minimum scope of work to develop a detailed, site-specific workplan (the specific elements of the workplan will be developed through negotiation with the monitoring Project Manager). If an agency of the Regional Response Team (RRT) decides to implement a bioremediation monitoring plan with its own resources, that agency will also need to develop such a workplan.

6.1 OBJECTIVES

The principal objectives of the monitoring program and the major elements of each objective are listed below.

Objective 1: Determine the efficacy of the selected bioremediation agent in enhancing the degradation of spilled contaminants.

- Certify the viability of microorganisms and/or nutrient composition of the bioremediation agent in a laboratory setting before or concurrent with the initial application; Done → this has been accomplished repeatedly
- Certify the ability of the bioremediation agent to degrade or enhance the degradation of spilled contaminants in a laboratory setting before or concurrent with the initial application; and Done → this has been accomplished repeatedly
- Determine the extent to which the bioremediation agent has enhanced the rate of contaminant biodegradation as compared to an untreated, contaminated site.

BP Deep horizon spill, within days of the waveland beach Mississippi demonstration in front of RRT IV DEQ DNR+ state Senators, the sandy beach, and marsh grass were free of detectable Hydrocarbons, whereas the areas next to the BP demonstration areas remained oiled!

Objective 2: Measure the environmental impact of bioremediation treatment for the duration of the monitoring activity. Dispersant toxicity tests with OSEII for the EPA show the oil toxicity is reduced by 1000 in 24 hours

- Determine the extent to which the bioremediation agent increases or decreases the toxicity of spilled contaminants; OSEII reduces
- Document adverse physical effects attributable to bioremediation agent application and monitoring activities; and in over 16,000 spill cleanups over 22 years there has never been ANY adverse effect with the use of OSEII
- Determine the extent to which the bioremediation agent alters the nutrient dynamics of the treated habitat. OSEII by causing oil to float prevents the depletion of O2 in the water column & keeps the oil & OSEII on the surface.

Objective 3: Ensure the comparability of data collected from all monitoring projects in the Region for use in a Region 6 bioremediation data base. OSEII really doesnot effect nutrient levels as tests have proven

- Employ standard methods and operating procedures at all monitoring projects; and
- Conduct sampling both at replicate treated and untreated (control) sites for all bioremediation activities, unless replication is specifically ruled out on the basis of informed judgement by the FOSC or the monitoring Project Manager. Sampling from replicate sites is needed to establish variance of means among sites.

6.2 USES OF COLLECTED DATA

The primary use of monitoring data will be for response management decision-making by the FOSC. Properly collected, validated, and interpreted data provide critical information to assess the efficacy and environmental impact of bioremediation treatment and related response activities. Such documentation is needed to identify and correct problems in the biological treatment process, to determine whether bioremediation end-points have been reached, to ensure that biotreatment is less environmentally harmful than the spilled pollutant, and to support cost recovery and other legal actions.

Secondarily, the data can also be used for developing regional and national data bases, interfacing with natural resource trustees, preparing interim and final reports, and revising this monitoring plan.

6.3 MONITORING PLAN DESIGN OSEII was successfully tested & monitored through EPA/NETAC Tiers III & IV

The monitoring plan described in this section is designed to be implemented in various levels of response based nominally on spill volume. The rationale for this design is that increasingly more comprehensive monitoring will be necessary and should be undertaken as the volume of a spill increases (assuming that the size of any bioremediation activity also increases), or as the potential for damage to sensitive resources attributable to the spilled oil or bioremediation activity increases, regardless of spill volume. (Weather conditions, the location of a spill, and the particular location of any ensuing bioremediation activity also need to be considered when determining the appropriate monitoring response level.) In addition, the design provides flexibility to tailor monitoring activities to best fit the conditions associated with a particular bioremediation activity.

Because a principal goal of monitoring is to establish whether the addition of bioremediation agents accelerates contaminant degradation without contributing significant adverse environmental impacts, the monitoring plan design provides for the comparison of data from replicate treated and untreated areas throughout the duration of a bioremediation activity. That is, the plan proposes that observations be made and samples collected and analyzed for: (1) uncontaminated, untreated source areas; (2) contaminated, untreated source areas; and (3) contaminated, treated source areas. This approach should be followed for each bioremediation activity and monitoring response level to the extent possible. after 16,000 spill cleanups, and numerous efficacy studies that by EPA & other governments OSE# has proven beyond a shadow of a doubt that OSE# accelerates contamination degradation without adverse effects to the environment or responders

Details of each monitoring response level and the criteria for selecting treated and untreated sites are provided below.

6.3.1 Monitoring Intensity Levels

Monitoring intensity levels describe the scale of field and laboratory activities that should be performed as part of the monitoring effort of a bioremediation activity. Monitoring intensity levels vary primarily with the size of the monitoring effort, rather than the specific types of activities to be performed. In this context, the "size" of the monitoring effort refers to the number of samples to be taken and the sampling density, as well as resource requirements needed to accommodate increased sampling and analysis activity.

The activities proposed for monitoring intensity levels assume that any bioremediation agent used is both listed on the National Oil and Hazardous Substances Pollution Contingency Plan (NCP) Product Schedule and has undergone controlled laboratory testing that at least demonstrates its ability to degrade oil. Generally, it is assumed that bioremediation agents have not undergone adequate field testing to demonstrate their efficacy and safety.

Intensity I Monitoring Response -- Spill <240 Barrels

Historically, oil spills smaller than 240 barrels (or 10,000 gallons) have been the most common. Bioremediation activities following a spill of less than 240 barrels could be undertaken, for example, to clean up habitats, such as sensitive marshes, where mechanical methods would be inaccessible or too disturbing to be practical, or to hasten cleanup of lightly-oiled shorelines outside of public-use areas. Monitoring of a bioremediation activity following an Intensity I spill should, at a minimum, incorporate the following activities:

Field Activities

Reconnaissance -- collection of screening and qualitative information through a preliminary survey of the spill area. Information collected will be used to assist in designating treatment and control sites, evaluating logistics of monitoring, and determining resource needs. Reconnaissance activities should include performing visual observation from aircraft or boat; tracking oil distribution and movement; assessing the presence, location, and abundance of spilled material; and evaluating potential logistical problems posed by the physical habitat. Generally, this type of information will be collected by the Shoreline Cleanup Assessment Team as part of the monitoring for the overall response to a spill. Therefore, reconnaissance for bioremediation monitoring should be coordinated

with these other information collection activities to minimize any unnecessary duplication of effort.

Environmental parameters assessment -- collection of information on weather conditions and measurement of field conditions or water quality, where applicable, to assist in selecting treatment and control sites and, later, in evaluating effects of bioremediation agent applications.

Sampling -- collection of water, sediment, and/or shoreline-material samples on which to perform laboratory efficacy and toxicity tests to evaluate effects of bioremediation agent applications, as well as nutrient balance analyses. Samples should be collected in triplicate at each sampling site, water depth (as appropriate), and time. For applications in marshes or shallow waters, surface water and the top two centimeters of sediment should be collected. For applications on beaches or shorelines, only shoreline material (e.g., sand, shell) needs to be collected. For applications on enclosed open water (such as bays), surface and bottom water samples should be collected to the extent practical; at a minimum, where water is less than 20 to 30 feet deep, pretreatment surface water samples and sediments should be collected.

Other visual observation and documentation -- collection of qualitative information on environmental characteristics to help evaluate the effects of bioremediation agent applications and ensure that the spill situation and ensuing response are documented thoroughly and completely. Visual observations and measurements should focus on assessing readily discernable effects of oil and oil plus bioremediation agent on indigenous biota; physical effects associated specifically with agent applications and monitoring activities; presence, location, and abundance of spilled material; appearance changes (such as percent change in color, stickiness, and consistency) in spilled contaminants and bioremediation agent materials; and distribution and movement of spilled contaminants and bioremediation agent materials. Documentation shall be collected and assembled over the period of the response.

Laboratory Activities

Efficacy testing -- use standard EPA-approved laboratory protocols or other validated and accepted methods to analyze collected samples to measure relative changes in the: (1) composition and amount of spilled oil to assess the effectiveness of bioremediation agent applications; and (2) nutrient concentration to assess effects of agent applications on ambient concentrations and the adequacy of the application strategy to maintain microbial growth and degradative activity.

Toxicity testing -- use standard EPA-approved laboratory protocols to analyze collected samples to assess and confirm the presence or absence of toxicological effects associated with bioremediation agent applications relative to those associated only with the spilled oil.

Intensity II Monitoring Response -- Spill 240 to 2,400 Barrels

As the size of a spill increases, the likelihood of adverse effects that are attributable to the spilled oil also increases. Consequently, it is possible that several distinct habitats or sensitive

resources may be threatened or affected by spilled oil and that bioremediation may be considered for treating more than one of them. The number of unique bioremediation activities and, particularly, the scale of any bioremediation activities that may follow a spill of 240 to 2,400 barrels (or 10,000 to 100,000 gallons) are likely to exceed those that may follow an Intensity I spill. The approach to monitoring should more carefully consider the potential merit of applying bioremediation agents in particular environments or of applying particular agents as part of the overall spill cleanup strategy. A two-phased approach is proposed.

Phase I Activities

This phase provides for a pilot or small-scale field test to be conducted with each unique bioremediation agent or for each distinct habitat proposed for treatment (depending on the scale of application planned and its potential effects). For example, a bioremediation activity to treat the entire area of a 500-barrel spill should be preceded by a small-scale field test. Alternatively, a bioremediation activity to treat only a one acre area of a 500-barrel spill that does not encroach on any sensitive resources would probably not require an initial field test.

Specific monitoring activities to be performed include the following, as defined above:

- Reconnaissance, results of which will be used to designate both the location and size of test plots (one-fifteenth the area proposed for full-scale treatment is suggested; however, the area may be larger depending on the overall size of the proposed treatment area);
- Sampling; and
- Efficacy and toxicity laboratory testing, focusing on analysis of trends over the test period.

Phase II Activities

Based on results of Phase I field applications and monitoring, bioremediation agents may be applied on a larger scale and to several oiled habitats. The types of monitoring activities conducted under this phase should be the same as those conducted for an Intensity I Monitoring Response. The monitoring regime should be repeated for each distinct habitat that is treated.

Intensity III Monitoring Response -- Spill >2,400 Barrels

A spill of this size may require a multiplication of the level of effort outlined for an Intensity II spill (i.e., several small-scale field tests -- one for each habitat considered for treatment or each bioremediation agent considered for use -- and several monitoring teams with appropriate equipment and supplies to collect samples and make observations). If the FOSC recommends and the RRT concurs that equipment, personnel, and financial resources needed to conduct recommended monitoring cannot be obtained, monitoring could be performed on fewer sites as long as these sites are representative of treated habitats and allow for appropriate controls. The same types of field and laboratory activities described for Intensity II monitoring should still be performed.

6.3.2 Selection of Treated and Untreated Sites

Treated and untreated (or control) sites should exhibit similar chemical and physical characteristics to support their comparability. Preferably, a number of unique treated and untreated sites should be selected for each significantly different habitat intended for bioremediation treatment. To select treatment and control sites, the following are among the criteria that should be considered: (1) environmental parameters; (2) physical habitat and geological morphology; and (3) oil loading and the probability of further oiling.

Chemical characteristics of the spill environment as well as temperature may influence the effectiveness of bioremediation treatment. For aquatic spills, whether in enclosed open water, coastal areas, or estuaries, try to ensure that the variability in the following environmental parameters between sites is no greater than indicated below:[12]

- Dissolved O_2 concentration -- ±2 to 3 ppm (should be ±1 ppm);
- Salinity -- ±3 to 5 ppt (should be ±1 to 3 ppt); and
- Temperature -- ±3 to 5°C (should be ±1 to 3°C).

The physical habitat and geological morphology of the spill area can affect: (1) the extent of contact between contaminants and potential microbial degraders; (2) the potential for contaminant or bioremediation agent migration from or into test areas; (3) the ease and success of agent application and sampling efforts; and (4) the potential for unexplainable variances in observation and sample analysis results. Potential variances between test areas attributable to wave action, tidal flushing, currents, boat traffic, and exposure to wind or other external forces also should be considered and minimized, where possible, in selecting test sites.

Because efficacy analyses focus on evaluating relative changes in the concentration of the constituents of oil between treated and untreated sites, it is important to ensure that: (1) uncontaminated source areas remain uncontaminated for the duration of the monitoring program; and (2) contaminated areas, upon selection, are similarly oiled, and are not re-oiled for the duration of the monitoring program (otherwise, monitoring will need to be re-initiated). Uncontaminated control areas should be carefully selected to minimize the potential of contamination. Booming of control areas may be helpful. The selection of contaminated areas should be restricted to those with uniform oiling (i.e., ±10 to 20% difference). To lessen the probability of further oiling of treatment or control areas, the selection of treatment and control source areas proximate to any of the following should be avoided if possible:

- Inflows of water or runoff;
- Petroleum discharge sources; and
- Marinas and fish camps.

[12] Suggested maximum variations for these environmental parameters were recommend by Jim Clark of EPA's environmental laboratory in Gulf Breeze, Florida, with concurrence of the Monitoring Workgroup of the Subcommittee on National Bioremediation Spill Response's Implementation Subgroup.

6.4 MONITORING PARAMETERS AND COLLECTION FREQUENCY

The environmental characteristics and measurements that should be assessed and the samples that should be taken as part of the field monitoring activities are presented in Exhibit 6-1, along with a schedule for performing these activities. Sampling at each site, water depth (as appropriate), and time should be performed in triplicate. Although the size of samples collected should be based on the requirements of the analytical methods to be used for their analysis, the sizes of 1 liter for water samples and 20 grams (or 20 milliliters) for sediment or shoreline-material samples are recommended minimums. All samples should be collected in methylene chloride-rinsed jars or bottles with teflon-lined caps, as appropriate.

Parameters and methods for performing laboratory analyses of samples collected are presented in Exhibit 6-2. Copies of analytical methods are provided in Appendix F. Other methods are currently being developed by the National Environmental Technology Applications Corporation in coordination with EPA.

6.5 DATA QUALITY REQUIREMENTS AND ASSESSMENTS

All data collection activities must be planned and conducted to produce data of known and acceptable quality. To help ensure that these objectives are meet, all contractors performing work as part of the monitoring effort must submit to EPA and the lead agency from the affected state a quality assurance plan. Parameters for defining data quality include precision, accuracy, representativeness, comparability, and completeness.

Representativeness and comparability have been designed into this monitoring plan through provisions for replicate sampling from treated and untreated areas and the use of standard, approved methods for sampling and laboratory analyses.

[DATA QUALITY REQUIREMENTS FOR EACH TYPE OF MEASUREMENT MADE DURING A BIOREMEDIATION ACTIVITY DEFINED BY PRECISION, ACCURACY, AND COMPLETENESS ARE TO BE DEVELOPED BY THE REGION]

6.6 SAMPLE CUSTODY PROCEDURES

Accurate identification and proper control of samples is important to help ensure the acceptability and usability of the resulting analytical data. Having standard sample custody procedures is particularly important where the individuals performing sample collection may vary and where individuals collecting samples will not be the ones analyzing the samples. Where the monitoring program is conducted by a contractor, the contractor should designate a sample custodian who will ensure that custody procedures are properly followed.

[SAMPLE CUSTODY PROCEDURES OUTLINING THE METHODS FOR IDENTIFYING AND TRACKING SAMPLES, VERIFYING PROPER LABELING OF SAMPLES, AND ARCHIVING SAMPLES ARE TO BE DEVELOPED BY THE REGION]

EXHIBIT 6-1

FIELD MONITORING PARAMETERS

Parameter	Sample Size[1]	Assessment/Collection Location	Assessment/Collection Frequency[2]
Visual observations (mortality, behavioral effects, appearance changes, oil distribution)	N/A	All test sites	Daily to the extent possible; at least each day that water, sediment, and/or shoreline material sampling is performed
Temperature (air, water)	N/A	All test sites	Days 0, 1, 4, 10, and 20
Salinity	N/A	All test sites	Days 0, 1, 4, 10, and 20
Dissolved oxygen	N/A	All test sites	Days 0, 1, 4, 10, and 20
Sea state	N/A	Activity area	Days 0, 1, 4, 10, and 20
Current	N/A	Activity area	Days 0, 1, 4, 10, and 20
Wind velocity	N/A	Activity area	Days 0, 1, 4, 10, and 20
Efficacy (water, sediment, and/or shoreline material)	1 liter water; 20 grams sediment or shoreline material	All test sites and, as appropriate, all water depths	Days 0, 4, 10, and 20
Toxicity (water, sediment, and/or shoreline material)	8 liters water; 20 grams sediment or shoreline material	All test sites and, as appropriate, all water depths	Days 0, 1, and 4

[1] N/A means "Not Applicable."
[2] Frequency is relative to the time of agent application.

EXHIBIT 6-2

LABORATORY ANALYSIS PARAMETERS

Parameter	Sample Matrix	Methodology	Recommended Methods
Oil hydrocarbons (C17, pristane, C18, phytane)	Water	GC + GC/MS	ASTM Method D3328
	Sediment/shoreline material	GC + GC/MS	ASTM Method D3328
NH_3	Water	Spectrophotometric	
	Sediment/shoreline material	Spectrophotometric	
NO_3	Water	Spectrophotometric	
	Sediment/shoreline material	Spectrophotometric	
NO_2	Water	Spectrophotometric	
	Sediment/shoreline material	Spectrophotometric	
PO_4	Water	Spectrophotometric	
	Sediment/shoreline material	Spectrophotometric	
Toxicity	Water	4-day acute or 7-day chronic	
	Sediment/shoreline material	4-day acute or 7-day chronic	

6.7 SAMPLING AND ANALYTICAL METHODS

All sampling and laboratory analyses should follow EPA or other approved methods, unless otherwise stipulated or requested by the FOSC.

[RECOMMENDED SAMPLING AND ANALYTICAL METHODS WILL BE PROPOSED AT A LATER DATE]

6.8 RESPONSE ORGANIZATION AND RESOURCE REQUIREMENTS

For federalized spills, the decision to use bioremediation is made in accordance with the National Oil and Hazardous Substances Pollution Contingency Plan (NCP) and the Region 6 Regional Contingency Plan (RCP). Once the decision and approval to bioremediate are final, the monitoring plan should be implemented. The RRT member agency that proposes the use of bioremediation on a particular spill will provide a Project Manager to implement the monitoring plan, subject to the approval of the FOSC. Personnel comprising monitoring teams and equipment resources to conduct monitoring will be provided by the RRT member agencies and contractors, as appropriate.

Specific responsibilities of the monitoring Project Manager include the following:

- Obtain approval from the FOSC for the monitoring plan;
- Assemble monitoring teams to perform observations and sampling, as appropriate, to successfully meet monitoring objectives;
- Coordinate all activities with the FOSC to ensure that monitoring does not interfere with other on-going or planned response operations;
- Name a sample custodian to coordinate all sample transfers and chain of custody;
- Ensure that monitoring teams have representation from each RRT member agency that wishes to participate;
- Provide a continuous communication link with the FOSC to ensure timely transfer of monitoring data and results that are relevant to response operations;
- Act as a liaison with natural resource trustees;
- Ensure that the quality of environmental data is known, documented, and sufficient to meet the requirements of the data users and decision makers; and
- Ensure the preparation and submission to the FOSC of all required reports on the monitoring effort.

Monitoring team members will be responsible for implementing this monitoring plan and any other bioremediation activity-specific procedures as directed by the Project Manager, ensuring

the quality of samples and data collected, and participating in the preparation and review of all required reports on the monitoring effort.

6.8.1 Personnel Requirements

The suggested minimum number of qualified personnel (in addition to the Project Manager) for carrying out the field activities associated with each monitoring response intensity level are listed below. Specific qualification requirements shall be provided by the RRT. In general, personnel responsible for making visual observations and measurements need to be trained or experienced in conducting physical observations in the field. Personnel responsible for collecting samples need to be properly trained and experienced in the collection of water, sediment, and shoreline material, as appropriate. Wherever possible, the same crews should conduct observations and sampling throughout the monitoring effort.

Intensity I Monitoring Response -- Spill <240 Barrels

- 2 people to conduct visual observations and appropriate documentation.
- 2 to 3 people to conduct land-based sample collection, as appropriate.
- 4 people to conduct water-based sample collection (2 boat operators and 2 sample collectors), as appropriate.

Intensity II and III Monitoring Responses -- Spills $\geq$240 Barrels

Personnel requirements for these levels of response will depend on the number and scale of each unique bioremediation activity undertaken simultaneously following a spill. The personnel requirements proposed for an Intensity I response should be used as a baseline and scaled-up as appropriate.

6.8.2 Minimum Equipment Requirements

RRT member agencies, other state agencies, and/or contractors that may oversee or participate in the monitoring for a bioremediation activity should be prepared to provide equipment resources necessary to conduct monitoring. The following equipment and supplies at a minimum should be assembled and be made ready for transport to the field to support a monitoring effort:

- Anemometers;
- Binoculars;
- Buckets (five-gallon size);
- Calculator;
- Camera (35 mm SLR) with film and appropriate filters;
- Cassette recorder (portable) with appropriate accessories;
- Cellular telephones and/or portable radios;
- Chain-of-custody forms;
- Chain-of-custody seals;

- Clipboard;
- Compass;
- Current meter;
- Field notebook;
- First-aid kit;
- Flashlight with batteries and spare bulb;
- Ice chest and ready access to ice;
- Kemmerer sampler or Van Dorn bottle, preferably stainless steel;
- Mercury thermometer (-5° to 45°C);
- Paper towels;
- Pens, pencils, and markers;
- Personnel safety equipment;
- Plastic sheeting and rubber bands;
- Polyvinyl chloride pipe, large diameter;
- Portable CTD or DO meter (or Winkler kit), pH meter, and conductivity meter (or refractometer));
- Resealable plastic bags;
- Sample containers (cubitainers, VOA vials, methylene chloride-rinsed one-liter jars, and methylene chloride-rinsed five-liter jars with silicone rubber "O" rings, silicone drain tubes, and teflon-lined lids);
- Sample preservatives;
- Shipping labels;
- Tape;
- Towels or rags;
- Video camera with tape, batteries, etc.;
- Vessels complete with communication and navigation equipment as appropriate for offshore motoring; and
- Watch.

6.9 DATA VALIDATION

All data will be subject to a thorough check by the FOSC and the monitoring Project Manager, or their designated representative, for errors in transcription, calculation, or computer input. In addition, the Project Manager will review all incident logs, sample logs, and data forms to ensure that requirements for documentation and data quality assessment have been met.

6.10 PERFORMANCE AND SYSTEM AUDITS

To help ensure that work being performed -- whether by contractor, EPA, or state personnel -- is progressing in accordance with the monitoring plan and any specified objectives or procedures, the FOSC, through the designated monitoring Project Manager, maintains the right to conduct performance or system audits of field and laboratory data collection activities. The category of audits are described below:

Management System Reviews -- evaluate the Quality Assurance Program of an organization, such as a firm contracted to conduct a monitoring project or laboratory sample analyses. The purpose of this review is to verify whether the quality assurance management procedures stated by contractor are in place, prior to a contract award.

Data Quality Audits -- evaluate a data set, or all data sets, of a particular project, by comparing the data set against specified data quality requirements for that data set.

Technical System Audits -- evaluate the actual environmental measurement data-collection systems and their associated quality control systems. These audits involve on-site auditing of field sampling activities, field measurement activities, and laboratory analytical procedures.

Performance Audits -- evaluate analytical methods and procedures of a laboratory. These audits are conducted by submitting performance evaluation samples to a laboratory for analysis. The samples contain specific pollutants in known matrices whose concentration and identity are unknown to the testing laboratory (the identity and concentration of pollutants is known to the submitter, however).

[PROVISIONS FOR PERFORMANCE AUDITS AND INTERNAL SYSTEM REVIEWS TO BE CONDUCTED BY THE MONITORING PROJECT MANAGER OR OTHER QUALITY ASSURANCE PERSONNEL ARE TO BE DEVELOPED BY THE REGION]

6.11 DOCUMENTATION AND REPORTING

During the course of a bioremediation activity and accompanying monitoring effort, the following reports should be prepared and submitted to the FOSC:

Activity reports -- provide descriptions of the bioremediation activity area, weather, unique observations, and activities undertaken, as well as the names, affiliations and signatures of persons on site. Activity reports should be prepared whenever activities on a site are undertaken.

Analytical reports -- provide laboratory analysis results of environmental and control samples. Analytical reports should be prepared and submitted by the analytical lab within 10 days after receipt of environmental samples for analysis.

After action report -- provides a description of the overall bioremediation activity and accompanying monitoring effort, including results of both field and laboratory activities. An interim draft should be submitted within 30 days after the end of the monitoring effort. A final draft (incorporating comments from the FOSC, RRT members, and other entities involved in the monitoring effort as well as photos) should be submitted within 60 days after submission of the interim draft. As to the discretion of the FOSC and the monitoring Project Manager, however, the time for submitting the final draft may vary depending on whether comments on the interim draft are received in a timely manner.

In addition, at the time the final after action report is submitted, all field notes, including those of contractors, should be submitted to the FOSC.

To facilitate information transfer and the development of a data base on bioremediation use and bioremediation agents, the Bioremediation Use Follow-up Form in Appendix G should be completed at the end of a bioremediation activity.

6.12 REVISING PLANS AND PROCEDURES

The monitoring plan and suggested procedures outlined in this section should be implemented and modified, as necessary, based on the cumulative experience and knowledge gained from conducting bioremediation field activities and associated laboratory activities. Recommendations for revisions should be submitted to the Region 6 RRT for approval. Upon approval by the RRT, revisions should be incorporated into the Region 6 RCP and other local contingency plans, as appropriate.

APPENDIX A

ADDITIONAL SOURCES OF INFORMATION ON BIOREMEDIATION

Chianelli, R.R., *et al.* "Bioremediation Technology Development and Application to the Alaskan Spill." *Proceedings: 1991 Oil Spill Conference.* March 4-7, 1991, San Diego, California, pp. 549-558.

Congress of the United States. *Bioremediation for Marine Oil Spills.* OTA-BP-O-70. Office of Technology Assessment, Washington, D.C. May 1991.

DeLune, R.D., R.P. Gambrell, J.H.Pardue and W.H.Patrick, Jr. "Fate of Petroleum Hydrocarbons and Toxic Organics in Louisiana Coastal Environments." *Estuaries.* 13(1), 1990, pp. 72-80.

Jordan, Randolph and James R. Payne. *Fate and Weathering of Petroleum Spills in the Marine Environment.* Michigan: Ann Arbor Science Publishers, Inc., 1980.

Lee, Richard F. "Metabolism of Petroleum Hydrocarbons in Marine Sediments." *Proceedings of a Symposium on Sources, Effects and Sinks of Hydrocarbons in the Aquatic Environment.* AIBS Publication, 1976.

Mearns, Alan J. *Observations of an Oil Spill Bioremediation Activity in Galveston Bay, Texas.* NOAA Technical Memorandum NOS OMA 57. June 1991.

Pitter, Pavel and Jan Chudoba. *Biodegradability of Organic Substances in the Aquatic Environment.* Boca Raton, Florida: CRC Press, 1990.

Pritchard, P. Hap and Charles F. Costa. "EPA's Alaska Oil Spill Bioremediation Project." *Environmental Science & Technology.* 25 (3), 1991, pp. 372-382.

Sauer, Ted and Paul Boehm. "The Use of Defensible Analytical Chemical Measurements for Oil Spill Natural Resource Damage Assessment." *Proceedings: 1991 Oil Spill Conference.* pp. 363-369.

REFERENCES

Anderson, J.P.E. "Principles of and Assay Systems for Biodegradation." *Biotechnology and Biodegradation*, vol. 4, Advances in Applied Biotechnology Series. Texas: The Portfolio Publishing Company, 1990, pp. 129-146.

Atlas, R.M. "Biodegradation of Hydrocarbons in the Environment," *Environmental Biotechnology*. New York: Plenum Press, 1988.

Atlas, Ronald M. "Microbial Degradation of Petroleum Hydrocarbons: an Environmental Perspective." *Microbiological Reviews*. March 1981, pp. 180-209.

Leahy, J.G. and R.R. Colwell. "Microbial Degradation of Hydrocarbons in the Environment." *Microbiological Reviews*. September 1990.

Portier, Ralph J. "Bioremediation Using Adapted Bacterial Cultures, Topic 1: Examination of Site Data and Discussion of Microbial Physiology With Regard to Site Remediation." *Proceedings of the Hazardous Materials Control Research Institute's First National Conference on Bioremediation*. 1990, pp. 321-361.

U.S. Environmental Protection Agency. *Guide for Preparation of Quality Assurance Project Plans for the National Estuarine Program*. EPA 556/2-88-001. Office of Marine and Estuarine Protection, Washington, D.C. June 1988.

APPENDIX B

BIOREMEDIATION USE AUTHORIZATION FORM

A. DETAILS OF SPILL -- TO BE PROVIDED AT TIME OF SPILL BY SPILLER (IF KNOWN), HIS AGENT, OR BY THE FOSC. ATTACH ADDITIONAL SHEETS IF NECESSARY.

1. SPILL DATA

a. Circumstances (fire, grounding, collision, etc.)

_____

_____

b. Location of spill (report all available details)

Distance and direction from nearest port _____

Latitude and longitude _____

Block (give reference) _____

Habitats affected _____

c. Time and date of spill _____

d. Potentially responsible party _____

Name of company _____

Address _____

Individual to contact _____

Telephone _____

e. Product spilled (the name or type of product spilled may be useful in establishing answers requested in Sections A.2 and A.3, below)

Type of product (crude oil or refined product) _____

Name of crude or product (if known) _____

Volume released (if known) _____

f. Type of release (instantaneous, continuous, intermittent, belching, etc.) _____

_____

g. Total potential volume of release (if still leaking) _____

2. PROPERTIES OF THE SPILLED OIL (IF KNOWN) (NOTE: General oil property information may be available from the files maintained as part of the Region VI Oil Spill Contingency Plan, Subpart H)

a. Specific gravity
or
API gravity _____

b. Viscosity, cp. _____ at temperature, °F _____

c. Pour point, °F _____

d. Sulfur content, %w _____

3. IS OIL EXPECTED TO BE AMENABLE TO BIOREMEDIATION?

- Easily _____
- Moderately _____
- With difficulty _____

How was this estimate made (e.g., from known oil properties, from constituent characteristics, from field trials, from laboratory tests, from historical use information)? _____

_____

_____

B. SPILL TRAJECTORY AND WEATHER -- TO BE PROVIDED AT TIME OF SPILL BY NOAA

(NOTE: Some of this information may be available from the spiller, the FOSC, or other interested parties.)

1. Weather conditions and forecast:

   a. Air temperature _____

   b. Wind speed _____

   c. Wind direction _____

   d. Visibility (in miles) _____

2. Sea conditions and forecast:

   a. Wave height, ft. _____

   b. Swell height, ft. _____

   c. Water depth, ft _____

   d. Salinity (if known) or possible presence of fresh water (e.g., from river runoff)

   _____

3. Currents - tidal and longshore

	Tidal		Longshore
a.	Speed, knots _____	c.	Speed, knots _____
b.	Direction _____	d.	Direction _____

4. Oil spill trajectory information -- forecasts should be made for at least 48 hours and preferably 96 or 120 hours.

   a. Surface trajectory forecast

   Expected position of center of spill (e.g., latitude and longitude coordinates) on

Day 1	_____	Day 4	_____
Day 2	_____	Day 5	_____
Day 3	_____		

NOTE: The leading edge of the spill may be as much as one to five miles in advance (downwind) of the center of the spill, depending on spill size, time, and wind speed.

Expected landfall (when, where, what habitats, and how much) _____

_____

What will be effects the on above if the winds change? _____

_____

What will be effect on above if the currents change? _____

_____

5. Spreading, weathering, dispersion

   a. Surface area of slick (see Table B.5.a in Subpart H of the Region 6 Regional Contingency Plan) at end of:

   Day 1 _____ Day 4 _____

   Day 2 _____ Day 5 _____

   Day 3 _____

   b. Amount lost by weathering, % (see Table B.5.b in Subpart H of the Region 6 Regional Contingency Plan) at end of:

   Day 1 _____ Day 4 _____

   Day 2 _____ Day 5 _____

   Day 3 _____

   c. Is emulsion (mousse) formation expected? (use Table B.5.c in Subpart H of the Region 6 Regional Contingency Plan to determine whether or not mousse formation should be expected) _____

   _____

   Immediately or after weathering? (see Table B.5.c in Subpart H of the Region 6 Regional Contingency Plan) _____

C. DETAILS OF BIOREMEDIATION PLAN -- TO BE PROVIDED AT TIME OF SPILL BY SPILLER (IF KNOWN), HIS AGENT, OR THE SSC

1. Specific location proposed for treatment _____

2. Bioremediation agent proposed for use

   Name _____

   Type of agent (nutrient or microbe) _____

   Is agent on the NCP Product Schedule? _____

   To what Tier-level has it been evaluated? _____

   Source of supply _____

   Estimated amount needed _____

   Amount available _____

3. Has testing demonstrated the agent's ability to degrade the substance spilled? _____

4. Equipment to be used for applying bioremediation agent (see Appendix C)

   Type (boat spray, backpack spray, sprinkler) _____

   Available from: Name _____

   Address _____

   Telephone _____

   Name of equipment (if known) _____

   _____

   Time (in hours) needed for transport to the spill site, after preparation

   _____

5. Has equipment been calibrated for use with bioremediation agents? _____

6. Planned rate of application _____ gal/acre

   How frequently will agent be reapplied? _____

7. What means will be used to guide the application?

_____

_____

8. Does a bioremediation application/operation plan exist?

_____

_____

9. Do special precautions need to be taken to prevent improper handling or application of the agent? If so, what are they? _____

_____

APPENDIX C

PERSONNEL AND EQUIPMENT RESOURCES IN REGION 6

[TO BE DEVELOPED BY THE REGION]

APPENDIX D

LABORATORY FACILITIES IN LOUISIANA AND TEXAS

Louisiana

Analytical and Environmental Testing Laboratory
1717 Seabord Drive
Baton Rouge, LA 70810
(504) 769-1930
FAX: (504) 767-0533

ENTEK Environmental Laboratory
14285 Airline Highway
Baton Rouge, LA
(504) 752-2900
FAX: (504) 756-2706

Environ-Med Labs, Inc.
1874 Dallas Drive
Baton Rouge, LA
(504) 928-0232

ETC/Toxicon
3213 Monterrey Drive
Baton Rouge, LA
(504) 925-5012
FAX: (504) 928-3840

SPL, Inc.
104 Guilbeau Road
Lafayette, LA
(318) 984-2374

Texas

Environmental Chemistry Lab
Lamar University
P.O. Box 10022
Beaumont, TX 77710
(409) 880-1773

Lower Neches Valley Authority
P.O. Box 3464
7850 Eastex Freeway
Beaumont, TX 77704
Mr. Dennis Becker
(409) 892-4011

Pan American Laboratory
5369 E. 14th Street
Brownsville, TX 78521
Ms. Mary Lipps
(512) 831-4266

Mega Laboratory
P.O. Box 3486
2702 Finfeather Road
Bryan, TX 77801
Mr. Bernie Camp
(409) 779-7680

Eastex Environmental Laboratory
P.O. Box 859
Coldspring, TX 77331
Mr. Mac Phillips
(409) 653-3249

Inter Mountain Laboratory
Route 3, Box 256 Highway 30
3304 Longmire
College Station, TX 77845
Mr. Bill Fielder
(409) 776-8945 or x4999

Core Laboratory
1733 Padre Island Drive
Corpus Christi, TX 78048
Mr. Chip Meadours
(512)289-2673

Gulf Coast Testing Laboratory
1205 N. Tancahua
Corpus Christi, TX 78401
Mr. Al Bell
(512) 882-5411

Jordan Laboratory
P.O. Box 2552
842 Cantwell Drive
Corpus Christi, TX 78403
Mr. Carl Crownover
(512) 884-0371

Fox Laboratory
P.O. Box 346
211 E. Monroe
Harlingen, TX 78550
Mr. David Humphrey
(512) 423-3196

ANACON, Inc.
9001 Airport Blvd. #605
Houston, TX 77061
(713) 941-6703

Edna Wood Laboratories, Inc.
4820 Old Spanish Trail
Houston, TX
(713) 747-7271

Microbiological & Biochemical Assay Labs (MBA Labs) (713) 928-2701
340 South 66th
Houston, TX

NDRC Laboratories, Inc. (713) 661-8150
1155 South Main
Houston, TX 77025

NUS (713) 488-1810
900 Gemini
Houston, TX

SPL (713) 660-0901
8880 Interchange Drive
Houston, Texas 77054

Water Quality Services Laboratory (713) 466-0958
17459 Village Green
Houston, TX 77040
Ms. Anne Fidelman

Environmental Services Laboratory (409) 298-4754
P.O. Box 992
1788 Highway 30
Huntsville, TX 77340
Mr. Bill Swain

Upper Guadalupe River Authority (512) 896-5445
P.O. Box 1278
215 West Water Street
Kerrville, TX 78029
Mr. Bob Hall

Analab Corporation (214) 984-0551
Route 2, Box 176 W
2600 Dudley Road
Kilgore, TX 75662
Mr. C. Whiteside and Mr. Bill Peery

TRA Laboratory (Lake Livingston) (409) 365-2292
P.O. Box 360
Livingston, TX 77351
Mr. Michael Knight

Southwest Research Institute
P.O. Box 28510
8500 Culebra Road
San Antonio, TX 78284
Mr. Oscar Saenez

(512) 684-5111

East Texas Testing Laboratory
1717 E. Erwin
Tyler, TX 75702
Ms. Toni Batton

(214) 595-6402

Angelina-Neches River Authority (409) 632-7795
P.O. Box 387
210 Lufkin Avenue
Lufkin, TX 75901
Mr. Kristy Lewis

Texas Environmental Services Lab (409) 727-6839
1045 Boston Avenue
Netherland, TX 77627
Mr. Don Budd

Sabine River Authority (409) 746-3284
P.O. Box 579
Owens-Illinois Road
Orange, TX 77631
Ms. Mary Vann

University of Texas at Port Aransas (512) 749-6711
P.O. Box 1267
Port Aransas, TX 78375
Dr. Robert Jones

Aqua Science-SK Laboratories (915) 658-1986
1122 S. Bryant Boulevard
San Angelo, TX 76903
Mr. Fred Teagarten

Pollution Control Laboratory (512) 734-9998
131 Bandera Road
San Antonio, TX 78228
Mr. Chuck Wallgren

Raba Kistner Laboratory (512) 699-9090
P.O. Box 690287
12821 W. Golden Lane
San Antonio, TX 78249
Mr. Frank Schwiezter

San Antonio River Authority Laboratory (512) 227-1373
P.O. Box 9284
100 E. Guenther Street
San Antonio, TX 78204
Mr. Mike Gonzales

San Antonio Testing Lab (512) 599-7670
4733 Rittiman Road
San Antonio, TX 78218
Mr. Richard Hawk

APPENDIX E

ACTION PLAN OUTLINE

[CURRENTLY UNDER DEVELOPMENT]

APPENDIX F

ANALYTICAL METHODS

[TO BE INCLUDED AT A LATER DATE]

APPENDIX G

INFORMATION FEEDBACK: BIOREMEDIATION USE FOLLOW-UP FORM

Lessons learned from a spill cleanup operation are most useful when others, particularly those not personally involved in the original cleanup operation, can benefit from them by drawing upon the original responders' experiences. Region 6 has established a program to facilitate the collection and transfer of information on uses of bioremediation that is intended to provide decision makers with case data upon which future decisions regarding bioremediation may be based. Particularly because response officials have very limited experience with bioremediation in uncontrolled environments, such as open water and other marine areas, this program is expected to be a valuable resource for supporting informed decisions regarding bioremediation.

The principal objective of this bioremediation information feedback program in Region 6 are as follows:

- To gather relevant, accurate, descriptive, and complete information from sites where bioremediation has been used for spill response; and

- To provide that information via an accessible network to future decision makers who are considering the use of bioremediation.

The Bioremediation Use Follow-Up Form on the following pages has been provided to guide information collection efforts in support of this program. A separate form should be completed for each unique bioremediation activity. Because certain information may not have been anticipated when the form was developed, feel free to provide any other information deemed appropriate regarding the use of bioremediation in a particular response action.

BIOREMEDIATION USE FOLLOW-UP FORM

A. SPILL INFORMATION

1. Spill event _____
2. Date _____
3. Location (e.g., offshore, wetlands, coastal) _____
4. Product(s) spilled _____
5. Amount of spill _____
6. Reason(s) for using bioremediation _____

_____

_____

7. Age of oil when bioremediation agents applied _____

B. BIOREMEDIATION AGENT INFORMATION

1. First Treatment or Application:

   a. Type of agent applied (e.g., nutrient, microbial, enzyme) _____

   b. Name of agent _____

   c. Agent listed on the NCP Product Schedule? _____

   d. Vendor _____

   e. Vendor address and phone number _____

   _____

   f. Rate effectiveness (compared to control site) on a scale of 1 to 10, 10 being the highest score

      Visual observation _____

      Oil chemistry _____

      Method used (e.g., GC, GC/MS, TPH) _____

2. Second Treatment or Application (complete if different from above):

   a. Type of agent applied (e.g., nutrient, microbial, enzyme) _____

   b. Name of agent _____

   c. Agent listed on the NCP Product Schedule? _____

   d. Vendor _____

   e. Vendor address and phone number _____

   _____

   f. Rate effectiveness (compared to control site) on a scale of 1 to 10, 10 being the highest score

   Visual observation _____

   Oil chemistry _____

   Method used (e.g., GC, GC/MS, TPH) _____

   _____

3. Third Treatment or Application (complete if different from above):

   a. Type of agent applied (e.g., nutrient, microbial, enzyme) _____

   b. Name of agent _____

   c. Agent listed on the NCP Product Schedule? _____

   d. Vendor _____

   e. Vendor address and phone number _____

   _____

   f. Rate effectiveness (compared to control site) on a scale of 1 to 10, 10 being the highest score

   Visual observation _____

   Oil chemistry _____

   Method used (e.g., GC, GC/MS, TPH) _____

C. SITE CONTROLS

1. Size and number of test site(s) _____

2. Size and number of control site(s) _____

3. Site security measures taken _____

_____

D. TREATMENT AREA LOCATION

1. On water (latitude and longitude) _____

2. Shoreline (latitude and longitude) _____

Shoreline type (e.g., sand, shell, cobble) _____

Shoreline zone (e.g., intertidal, surge, storm/overwash) _____

Depth of shoreline oiling _____

E. APPLICATION INFORMATION

1. Microbial counts before application _____

2. Microbial counts after application _____

3. Applications performed by (names and titles) _____

_____

4. Application method(s) used _____

_____

5. Application date(s) _____

6. Application conditions (e.g., winds, waves) _____

7. Agent concentration and rates (e.g., gal/acre) _____

8. Additional information on re-applications _____

_____

F. MONITORING

1. Schedule and duration (e.g., weekly for 3 months) _____

_____

2. Method (e.g., foot, by air, boat) _____

3. Monitoring performed by (names and titles) _____

_____

4. Toxicity noted _____

G. PROBLEMS ENCOUNTERED (e.g., weather, site security, application)

_____

_____

_____

H. LESSONS LEARNED

_____

_____

_____

_____

I. CONTACTS

1. OSC (name, address, and phone) _____

_____

2. SSC (name, address, and phone) _____

_____

3. Form completed by (name, title, and agency) _____

_____